

The distribution of carotenoids in hens fed on biofortified maize is influenced by feed composition, absorption, resource allocation and storage

Jose Antonio Moreno ^{1,*}, Joana Díaz-Gómez ^{1,2,*}, Carmina Nogareda ¹, Eduardo Angulo ¹, Gerhard Sandmann ³, Manuel Portero-Otin ⁴, José C E Serrano ⁴, Richard M Twyman ⁵, Teresa Capell ⁶, Changfu Zhu ⁶, and Paul Christou ^{6,7}

Corresponding author contact details: Paul Christou, Department of Plant Production and Forestry Science, ETSEA, University of Lleida-Agrotecnio Center, Av. Alcalde Rovira Roure, 191, 25198 Lleida, Spain. Tel. +34 973 702831. E-mail christou@pvcf.udl.es

Supplementary Material

Supplementary Table 1. Feed carotenoid composition¹.

Carotenoids	WT ²	HC ³	BKT ⁴	COM ⁵
Neoxanthin (µg/g)	ND ⁶	0.46±0.05	ND	0.12±0.01
Violaxanthin (µg/g)	0.07±0.01 ^a	3.25±0.45 ^b	2.25±0.27 ^b	0.63±0.15 ^a
Lutein (µg/g)	0.18±0.02 ^a	3.08±0.38 ^b	0.45±0.22 ^a	2.97±0.36 ^b
Zeaxanthin (µg/g)	0.36±0.03 ^a	12.41±3.1 ^b	2.8±0.43 ^a	4.39±0.41 ^a
α-Cryptoxanthin (µg/g)	ND	5.38±0.59 ^a	0.2±0.03 ^b	0.41±0.05 ^b
β-Cryptoxanthin (µg/g)	0.03±0.01	3.29±0.39 ^a	0.56±0.10 ^b	0.56±0.05 ^b
β-Carotene (µg/g)	0.2±0.01	3.18±0.42 ^a	1.04±0.18 ^b	0.12±0.02 ^b
Astaxanthin (µg/g)	ND	ND	4.42±0.70	ND
Other ketocarotenoids (µg/g)	ND	ND	2.09±0.32	ND
PVA	0.23±0.01	6.47±0.81	1.60±0.28	0.68±0.07
Non-PVA	0.61±0.06	24.58±4.57	5.7±0.95	8.52±0.98
Total	0.84	31.05	13.81	9.2

¹ Values shown are the mean and standard error for each treatment (n = 5 **biological replicates**). Means within a row lacking a common superscript differ (p < 0.05)

²WT, diet supplemented only with white maize (wild type)

³HC, diet supplemented with genetically engineered maize enriched in carotenoids

⁴BKT, diet supplemented with genetically engineered maize enriched in ketocarotenoids

⁵COM, diet supplemented with standard commercial yellow maize

⁶ND = not detected

Supplementary Table 2. Carotenoids in egg yolks (µg/g fd) on day 32 of the trial (day 20 of experimental diet)¹.

Carotenoid (µg/g)	WT ²	HC ³	BKT ⁴	COM ⁵
Neoxanthin	0.02±0.01 ^a	1.75±0.15 ^b	ND ⁶	0.09±0.02 ^a
Violaxanthin	0.03±0.01 ^a	2.91±1.11 ^b	5.38±0.52 ^b	0.31±0.07 ^a
Lutein	1±0.47 ^a	5.93±1.22 ^b	1.01±0.11 ^a	4.64±0.8 ^b
Zeaxanthin	0.6±0.19 ^a	29.89±2.09 ^b	10.77±1.33 ^c	5.72±1.03 ^{ac}
α-Cryptoxanthin	0.03±0.01 ^a	13.93±0.58 ^b	0.31±0.03 ^a	0.45±0.09 ^a
β-Cryptoxanthin	0.08±0.02 ^a	2.64±0.13 ^b	0.37±0.06 ^a	0.28±0.04 ^a
β-Carotene	0.05±0.02 ^a	0.45±0.02 ^b	0.09±0.01 ^a	0.05±0.01 ^a
Astaxanthin	ND	ND	6.56±0.56	ND
Other ketocarotenoids	ND	ND	1.69±0.21	ND
PVA	0.13±0.04	3.09±0.15	0.46±0.07	0.33±0.05
Non-PVA	1.68±0.69	54.41±5.15	17.47±1.99	11.21±2.01
Total	1.81	57.5	26.18	11.54

¹Values shown are the mean and standard error for each treatment (n = 5 **biological replicates**). Means within a row lacking a common superscript differ ($p < 0.05$)

²WT, diet supplemented only with white maize (wild type)

³HC, diet supplemented with genetically engineered maize enriched in carotenoids

⁴BKT, diet supplemented with genetically engineered maize enriched in ketocarotenoids

⁵COM, diet supplemented with standard commercial yellow maize

⁶ND = not detected

Supplementary Table 3. Carotenoids in liver (µg/g fd) at day 32 (20th day of feed treatment) of the experiment.

Carotenoid	WT ²	HC ³	BKT ⁴	COM ⁵
Violaxanthin (µg/g)	ND	0.55±0.15 ^a	ND	0.05±0.01 ^b
Lutein (µg/g)	1.13±0.3 ^b	9.2±2.59 ^a	0.24±0.05 ^b	4.0±0.95 ^{ab}
Zeaxanthin (µg/g)	1.39±0.37 ^b	14.04±3.96 ^a	1.6±0.38 ^b	0.72±0.19 ^b
α-Cryptoxanthin (µg/g)	ND	1.62±0.45	1.22±0.32	0.56±0.16
β-Cryptoxanthin (µg/g)	ND	0.81±0.23	ND	0.51±0.14
β-Carotene (µg/g)	ND	ND	0.65±0.08	ND
C450*	ND	ND	1.45±0.31	ND
Astaxanthin (µg/g)	ND	ND	0.82±0.11	ND
Other ketocarotenoids (µg/g)	ND	ND	0.66±0.11	ND
R350**	ND	121.4±24.9 ^a	41.6±6 ^b	ND
Retinol	380±74 ^b	1397±218 ^a	1790±308 ^a	1454±236 ^a
PVA	0	0.81±0.23	0.65±0.08	0.51±0.14
Non-PVA	2.52±0.67	25.41±7.15	3.06±0.75	5.33±1.31

*C450 most likely β-carotene-5,6-epoxide or β-carotene-5,6,5',6'-diepoxide; tr trace amounts below 0.01;. *R350 is regarded as a non-polar retinoid-related product with an absorbance spectrum showing three distinct peaks at 328, 348, 370 nm

¹Values shown are the mean and standard error for each treatment (n = 5 **biological replicates**). Means within a row lacking a common superscript differ (p < 0.05)

²WT, diet supplemented only with white maize (wild type)

³HC, diet supplemented with genetically engineered maize enriched in carotenoids

⁴BKT, diet supplemented with genetically engineered maize enriched in ketocarotenoids

⁵COM, diet supplemented with standard commercial yellow maize

⁶ND = non detected